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Research Article

A COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF THE ETHANOLIC EXTRACTS OF *PHLOGACANTHUS THYRSIFLORUS, HOTTUYNIA CORDATA, CURCUMA CAESIA* AND *SYZYGIUM JUMBOS*

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ABSTRACT

The study was conducted to know and compare the antibacterial activity of the ethanolic extracts of *Phlogacanthus thyrsiflorus (EEPT), Hottuynia cordata (EEHC), Curcuma caesia (EECC)* and *Syzygium jumbos (EESJ)* on Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa by disc diffusion method. The ethanolic plant extracts were prepared from the bark of *Syzygium jumbos* and leaves of *Phlogacanthus thyrsiflorus, Hottuynia cordata* and *Curcuma caesia* by percolation method. Clinical isolates of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were obtained from the Department of Microbiology, Assam Medical College & Hospital. Disc diffusion method for antimicrobial susceptibility testing was performed according to the standard Kirby Bauer method. The whatmann-1 filter paper discs of 6mm sizes impregnated with the plant extracts were placed on Mueller-Hinton agar plates seeded with bacterial cultures of 0.5 Mc Farland standards. Ciprofloxacin (5µg/disc) was used as positive control. The antibacterial activities were assessed by the presence or absence of inhibition zones after incubating the plates at 37^0c for 24 hours. The ethanolic extracts of *Phlogacanthus thyrsiflorus (EEPT) and Hottuynia cordata (EEHC)* showed significant anti-bacterial activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Ethanolic extracts of *Syzygium jumbos (EESJ)* showed significant activity against Staphylococcus aureus and Escherichia coli while ethanolic extracts of *Curcuma caesia (EECC)* showed significant anti-bacterial activity against Staphylococcus aureus. Maximum zones of inhibition to Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were exhibited by *Phlogacanthus thyrsiflorus*, *Syzygium jumbos* and *Phlogacanthus thyrsiflorus* respectively.

Key words: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, disc-diffusion method.

INTRODUCTION

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine[1]. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects[2]. Moreover antibiotic resistance has become a global concern in recent years, especially in developing countries like India because infectious diseases are one of the major causes of mortality in these countries[3]. During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased astronomically which is no longer matched by expansion in the arsenal of agents available to treat infections[4]. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore[2].

Four medicinal plants, which have been used as folk medicine for common infections in the north-east India, were selected for this research, to study the antibacterial activity of their ethanolic extracts against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. These plants included *Phlogacanthus thyrsiflorus, Hottuynia cordata*, *Curcuma caesia* and *Syzygium jumbos.*

Aqueous and acetone extracts of bark of *Syzygium jambos* has been screened for antimicrobial activity earlier[5-6]. But the antibacterial activity of ethanolic extracts of the leaves *Phlogacanthus thyrsiflorus, Hottuynia cordata*, *Curcuma caesia* and the bark of *Syzygium jambos* has not been evaluated so far. In this study an attempt has been made to evaluate and compare the antibacterial effects of these plants by in vitro disc diffusion test.

Phlogacanthus thyrsiflorus, a gregarious shrub, belonging (Acanthaceae) is commonly known as Teeta phool in Assamese and Lal basak in Bengali and Hindi. It is used in curing coughs, cold, chronic bronchitis, asthma and rheumatism. Fruits and leaves are taken by the Karbi tribes of Assam after burning them as a specific treatment for fever. It is used as an anti-allergic. Flowers are antidote to pox, prevents skin diseases like sore, scabies etc[7].

Houttuynia cordata Thunb. (Saururaceae) is a perennial herbaceous plant. In the traditional medicines of Naga tribes of Northeast India, the leaves of *Houttuynia cordata* are used by the natives as a popular cure against intestinal helminthic infections[8]. It also has been used for the treatment of chronic sinusitis and nasal polyps[9].

Curcuma caesia Roxb., (Zingiberaceae), is popularly known as Kali haldi in India[10]. It is a perennial herb with bluish black rhizome, native to North-East and Central India. The rhizomes are used in the treatment of hemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorders, as anthelminthic, as aphrodisiac, in inflammation and gonorrhoeal discharges[11].

Syzygium jambos (L) is generally a tree reaching 7.5-12 m in height. Its bark is traditionally used to treat infectious diseases. Aqueous and ethyl acetate extracts of *S. jambos* leaves have been shown to posses anti-inflammatory activity in adjuvant carrageenan induced inflammation model in rats. An infusion of the fruit acts as a diuretic and sweetener. Preparation of the flowers is believed to reduce fever[5].

MATERIALS AND METHODS

Collection of plant material and preparation of ethanolic extract

Fresh leaves of *Phlogacanthus thyrsiflorus, Hottuynia cordata* and *Curcuma caesia* and bark of *Syzygium jambos* were used in the present study. The materials were collected from the Assam Medical College & Hospital [AMCH] campus. Plant materials were authenticated by Dr. M. Islam, Professor of Life sciences, Dibrugarh University, Assam. A voucher specimen (No. DU/LS/251) was deposited at the Department of Life Sciences, Dibrugarh University. The cleaned materials were dried in shade, grinded to fine powder with the help of a mixer grinder and ethanolic extracts were prepared using 90% ethanol by percolation method[12].The extracts were filtered and allowed to evaporate till dryness. Each extract was transferred into clean and dried airtight vials and stored at 2^0-8^0C until ready for use.

Microorganisms

Pathogenic bacterial isolates of *Staphylococcus aureas*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Dept. of Microbiology, AMCH. The organisms were sub cultured and stored in a semisolid medium at 4^0C until needed.

Preparation of the media

3.7% of Muller Hinton Agar was mixed with hot distilled water and autoclaved at 15 lb pressure for 15 minutes. After autoclaving, it was allowed to cool to 45^0C-50^0C. Then the medium was poured into sterilized Petri dishes with a uniform depth of approximately 4 mm.

Preparations of plant extract impregnated discs

Whatman no.1 filter paper was used to prepare discs of 6 mm in diameter. They were sterilized by autoclaving and subsequently dried at 80°C for an hour in a hot air oven. The discs were then impregnated with the ethanolic extracts of *Phlogacanthus thyrsiflorus, Hottuynia cordata*, *Curcuma caesia* and *Syzygium jambos* to get the final concentration of 1mg/disc and 0.5mg/disc for each plant extract respectively. The plant extract impregnated discs were then dried and then kept in sterile condition till further use.

Disc diffusion method

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of antimicrobial activities of plant extracts[13]. A bacterial suspension adjusted to 0.5 Mc Farland standard (1.5×10^8 CFU/ml) was used to inoculate Muller Hinton agar plates evenly using a sterile swab. The plates were left ajar for 5 minutes and then the discs impregnated with the four plant extracts were placed individually on the Muller Hinton agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. The discs were spaced far enough to avoid both reflections waves and overlapping rings of inhibition.

A standard commercial disc of Ciprofloxacin (5µg/ml) was used as a standard reference and an ethanol (90%) impregnated disc was used as a negative control in each case. Each test plate contained 6 discs one of which was a positive control i.e., a standard commercial antibiotic disc (Ciprofloxacin 5µg/disc) and a negative control i.e., ethanol impregnated disc. Besides the controls, each plate had four different plant extract impregnated discs placed about equidistant to each other. The plate was then incubated at 37°c for 24 hours in inverted position to look for zones of inhibition to ascertain antibacterial activity of the ethanolic plant extracts against selected micro organisms.

Zones of inhibitions produced by the sensitive organisms were demarcated by a circular area of clearing around the plant extract impregnated discs and were compared with the zone of inhibition produced by the positive control(ciprofloxacin 5µg/disc) and the negative control (ethanol impregnated disc). The tests were repeated six times to ensure reliability.

TABLE 1: ZONES OF INHIBITIONS AS SHOWN BY ETHANOLIC PLANT EXTRACTS AT DIFFERENT CONCENTRATIONS AGAINST SELECTED MICROORGANISMS

Extracts/ Positive control	EEPT		EEHC		EECC		EESJ		Ciprofl-oxacin
Concentration	1mg/ disc	0.5mg/disc	1mg/ disc	0.5mg/disc	1mg/ disc	0.5mg/ disc	1mg/ disc	0.5mg/disc	5µg/ disc
Microorganisms	Zones of inhibition (mm)								
Staphylococcus aureus	17.67± 0.666	14.0 ± 0.577	16.83± 0.60	13.50± 0.562	16.17± 0.909	12.67± 0.614	15.33± 0.494	12.17± 0.600	21.83± 0.807
Escherichia coli	16.17± 0.477	14.50± 0.428	14.50± 0.763	11.67± 0.494	-----	------	17.50± 0.428	14.69± 0.494	22.17± 0.472
Pseudomonas aeruginosa	15.83± 0.477	12.00± 0.365	15.00± 0.577	12.33± 0.557	-----	------	-----	-----	20.17± 0.60

Data represents Mean ± Standard error of mean (n = 6).

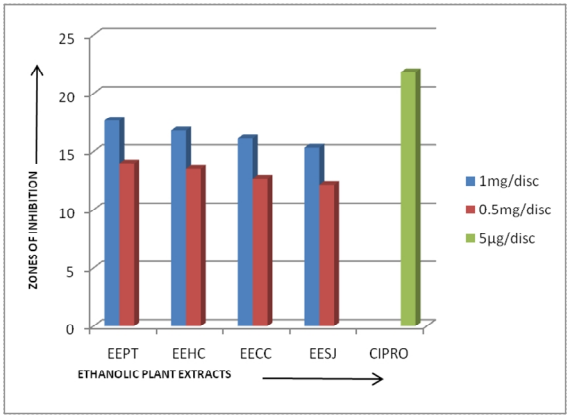

Figure 1: Antibacterial activity of ethanolic plant extracts against Staphylococcus aureus at different concentrations.

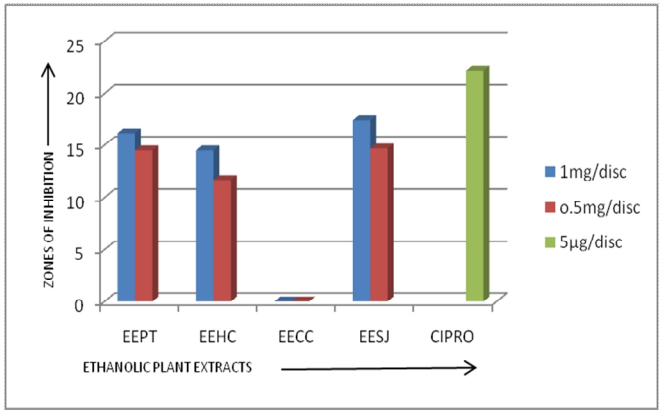

Figure 2: Antibacterial activity of ethanolic plant extracts against Escherichia coli at different concentrations.

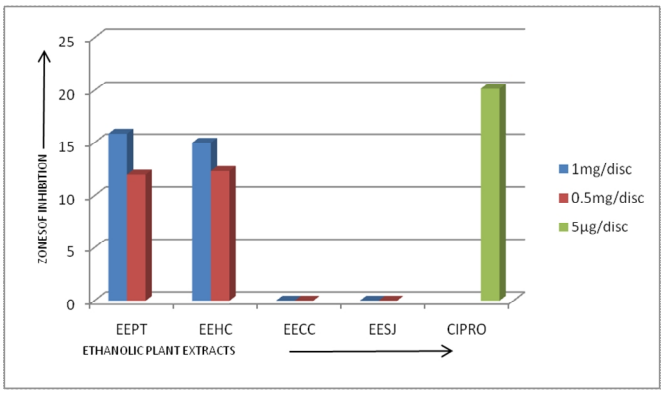

Figure 3: Antibacterial activity of ethanolic plant extracts against Pseudomonas aeruginosa at different concentrations.

RESULTS

After the incubation period, the zones of inhibition produced by the sensitive organisms selected for the study were measured using calipers and recorded. In the study positive control was found to produce zones of inhibition against all selected microorganism but the bacteria were insensitive to the negative control (ethanol impregnated disc); so no zone of inhibition was noted for the negative control. The zones of inhibitions as shown by ethanolic plant extracts and the positive control at different concentrations against the selected microorganisms are depicted in table 1 and the comparative activity of the plant extracts against the selected individual microorganism is represented with bar diagrams in figure 1, 2 and 3.

DISCUSSION

The ethanolic extracts of leaves of *Phlogacanthus thyrsiflorus, Hottuynia cordata, and rhizome of Curcuma caesia and bark of Syzygium jumbos* were investigated individually for antimicrobial activity by disc diffusion method against clinical strains of Staphylococcus aureas, Escherichia coli and Pseudomonas aeruginosa. For both the concentrations of the plant extract impregnated discs (i.e. 1mg/disc & 0.5mg/disc), the ethanolic extract of *Phlogacanthus thyrsiflorus* showed considerably high activity against Staphylococcus aureas and Escherichia coli than other extracts. *Phlogacanthus thyrsiflorus* and *Hottuynia cordata* showed antibacterial activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. *Syzygium jumbos* showed antibacterial activity against Staphylococcus aureus and Escherichia coli. *Curcuma caesia* showed antibacterial activity against Staphylococcus aureus. Maximum zones of inhibition to Staphylococcus aureus was exhibited by *Phlogacanthus thyrsiflorus*. Maximum zones of inhibition to Escherichia coli were exhibited by *Syzygium jumbos*. *Phlogacanthus thyrsiflorus* exhibited maximum activity against Pseudomonas aeruginosa. This probably explains the use of these plants by indigenous people of the north-east India against a number of infections since generations.

The variation in the effectiveness of the extract against different microorganisms may be attributed to the phytochemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. It has been suggested that the antimicrobial activity is mainly due to the presence of essential oils, flavanoids and terpenoids, alkaloids, tanins, saponins and other natural polyphenoilc compounds or free hydroxyl groups in plant extracts[14-16].

Presence of flavonoids and saponins in *Phlogacanthus thyrsiflorus*[7], flavonoids and volatile oils in *Hottuynia cordata*[18], flavonoids in *Syzygium jumbos*[6] and tannin, saponins and flavonoids in *Curcuma caesia*[11] have been detected in some studies. Presence of alkaloids in *Phlogacanthus thyrsiflorus*, *Hottuynia cordata, Syzygium jumbos* and *Curcuma caesia* was ascertained in the department of pharmacology, AMCH using Wagner's test which may add to their antibacterial effects[19].

A synergistic relationship between antioxidant status and antimicrobial effects of the plants are coming into light nowadays. Belofsky et al. demonstrated an increase in the antimicrobial activity of pure compounds when they are combined with antioxidants. Therefore, we can consider that if both antimicrobial and antioxidant compounds exist in the extracts, they could interact and enhance the antimicrobial activity[20]. Antioxidant effects of plant extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes[21]. Flavonoids in particular have been shown to have potent antioxidant and have free radical scavengering activity[22]. These compounds are also present in the plant extracts selected for this study as described above, which may add to their antibacterial effects.

Particular reference may be made for the use of ethanolic extracts of the plants for this study, because it has been observed that ethanol can extract higher amount of polyphenolic compounds and more bioactive flavonoid compound. Nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results[19].

CONCLUSION

The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative for treatment of infections. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

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